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Volatile Elements of Coconut Toddy (*Cocos Nucifera*) by Gas Chromatography–Mass Spectrometry

Karthikeyan R*, Suresh Kumar K, Singaravadivel K and Alagusundaram K

Department of Food Microbiology, Indian Institute of Crop Processing Technology, Ministry of Food Processing Industries, Govt. of India, Thanjavur (TN), India

Abstract

Volatile components of coconut toddy were analyzed using GC Clarus 500 Perkin Elmer (Germany)-MS and identified by matching the mass spectra obtained with those present in the "NIST 2005" Library. Totally Thirty one volatile components were identified and were different quantities amounts of volatile components. The highest peak of volatile components in the fresh toddy observed was Lupeol and Squalane.

Keywords: Volatile components; Coconut toddy; *Cocos nucifera*

Introduction

Toddy is one of the traditional, social and local drink extracted from either a coconut tree or a Palm tree Spadix. In the states of Tamil Nadu and Kerala, it is known as "Kallu", Indonesia known as nira, Sri Lanka as toddy, Thailand as maprau, North Africa as lagbi. The fermented beverages are those which are produced from fruit juice or plant sap by natural fermentation. In India, wild dates (Phoenix sylvestris), coconut palm (*Cocos nucifera*), palmyara (*Borassus flabellifer*) etc. are frequently used for this purpose. Toddy contains 4-5% of alcohol [1-3].

The methods of tapping the coconut palm are very ancient. When the palm has reached the normal bearing stage, every leaf axis produces a spadix or inflorescence. This unopened flower (spathe) is prepared by slightly brushing it with gentle taps of small mallet. To prevent it from opening, the spathe is slightly bound round with fibre. When the spathe is nearly ready to produce toddy, which is after about three weeks, about two to three inches is cut from the end. During the preparation the spathe is gradually bend over, so that by the time toddy flows, a receptacle can be placed at the end for its collection. The flow of juice increases gradually and the pot should be changed twice daily, at the same time shaving a thin slice from the end of the spathe, tapping slightly with the mallete and smearing on a mixture of bruised leaves for stimulating the flow of toddy from the spathe [1]. By the time collection of the sap is over, fermentation sets-in immediately and the sap is converted into fresh toddy or sweet toddy. This product is a milky white and effervescent.

Fresh toddy is a sweet, oyster white and translucent fluid and it is considered to be as pure as mothers' milk and traditionally believed that it has many medicinal properties and a refreshing health drink. If it is kept undisturbed, fermentation continues, which results in the production of acetic acid. A characteristic smell in the fresh toddy changed to fermented acid smell. To collect systematic data available on the volatile chemical and biological composition of toddy this study was conduct.

Materials and Methods

Materials

Toddy was obtained from the coconut plantation in Thanjavur, Tamil Nadu, India. The samples were taken at random after morning and afternoon tapping.

Methods

50 ml of toddy sample was taken into the separating funnel and 50 ml of organic solvent was added to same. The flask was stoppered tightly and shaken well. The gas produced inside the separating funnel was released at successive intervals by opening the knob. The shaking was done until there was less pressure inside the flask. The flask was kept undisturbed for 10 minutes in a stand. Separation of two layers inside the flask was observed. The aqueous layer was drained out. To the organic layer 25 ml of the organic solvent was added. Then shaking and draining was done as such in the first step. The organic layer was concentrated to about 1–2 ml by giving nitrogen flushing to the sample. Then it was filtered through a Whatmann filter No. 4 paper containing sodium sulphate to get clear solution. Then it was injected into the GC-MS for analysis [3].

The GC-MS analyses of the extracts were performed using a set of GC-MS, GC Clarus 500 Perkin Elmer (Germany) with Elite-5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30×0.25 mm×0.25 µm df column of 110°C -2 min hold at temperatures. The injector temperature was 250°C with mode split 10:1. Helium was used as a carrier gas at a pressure of 12 psi and the ion source working in electron impact(EI) mode at 70 eV was held about 200°C [3,4].

The identification was done by matching the mass spectra obtained with those present in the "NIST 2005" Library. The internal standard used for quantification of volatile using GC-MS was 1, 4–dichlorobenzene [5,6].

A method was standardized using different organic solvents viz., Chloroform Dichloromethane, Diethyl ether, chloroform, for the extraction of phytochemical compounds present in the Toddy sample. The extract from diethyl ether gave few expected compounds like Ethyl hydrogen succinate, Hydroquinone, Phenyl ethyl Alcohol, 2,4,6,8-Tetraazabicyclo [3.3.0] octan-3-one, 7-nitroimino, Dibutyl phthalate, Oleic acid, Sqyalene and n-Hexadecanoic. The extract

***Corresponding author:** Karthikeyan R, Department of Food Microbiology, Indian Institute of Crop Processing Technology, Ministry of Food Processing Industries, Govt. of India, Thanjavur-613 005 (TN), India, Tel: +919894098168; E-mail: krbiotech@gmail.com

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S.No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	6.05	Ethyl hydrogen succinate	$C_6H_{10}O_4$	146	32.44
2	9.19	2-Buten-1-ol, propanoate	$C_7H_{12}O_2$	128	0.37
3	11.63	Dichloroacetic acid, 2,2-dimethylpropyl ester	$C_7H_{12}Cl_2O_2$	198	0.46
4	14.25	3,4-Hexanediol, 2,5-dimethyl	$C_8H_{18}O_2$	146	0.46
5	15.08	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	0.93
6	16.43	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	1.85
7	16.57	Nonanoic acid	$C_9H_{18}O_2$	158	4.63
8	17.16	3-Pentanol, 2-chloro-4-methyl-, (R*,S*)-(ñ)-	$C_6H_{13}ClO$	136	1.39
9	18.58	3-Pentanol, 2,3-dimethyl-	$C_7H_{16}O$	116	1.85
10	19.26	Pentanoic acid, 10-undecenyl ester	$C_{16}H_{30}O_2$	254	18.54
11	25.19	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	23.17
12	29.58	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	13.90

Table 1: Compounds identified by GC-MS in Dichloromethane extract.

S.No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	6.07	Aminocyanoacetic acid	$C_3H_4N_2O_2$	100	0.41
2	10.60	Butanoic acid, 2-oxo-, methyl ester	$C_5H_8O_3$	116	0.00
3	14.11	à-D-Mannopyranoside, methyl 3,6-anhydro-	$C_7H_{12}O_5$	176	0.00
4	16.56	Propanedioic acid, propyl-	$C_6H_{10}O_4$	146	0.51
5	19.24	2,3-Epoxyhexanol	$C_6H_{12}O_2$	116	1.02
6	25.20	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	3.05
7	29.63	Squalene	$C_{30}H_{50}$	410	41.16
8	33.91	Lupeol	$C_{30}H_{50}O$	426	53.86

Table 2: Compounds identified by GC-MS in Chloroform extract.

S.No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	2.74	2,4,6,8-Tetraazabicyclo[3.3.0] octan-3-one, 7-nitroimino	$C_4H_6N_6O_3$	186	16.31
2	3.59	Hexanoic acid	$C_6H_{12}O_2$	116	2.95
3	5.38	Phenylethyl Alcohol	$C_8H_{10}O$	122	12.67
4	6.12	Ethyl hydrogen succinate	$C_6H_{10}O_4$	146	33.61
5	7.69	Hydroquinone	$C_6H_6O_2$	110	34.35
6	8.73	n-Decanoic acid	$C_{10}H_{20}O_2$	172	0.35
7	16.42	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.43
8	16.56	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.20
9	19.27	Oleic Acid	$C_{18}H_{34}O_2$	282	4.53
10	23.91	Squalene	$C_{30}H_{50}$	410	1.31
11	25.22	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	0.59

Table 3: Compounds identified by GC-MS in Diethyl ether extract.

from chloroform revealed the presence of the following compounds like Aminocyanoacetic acid, Lupeol, Squalene. Dichloromethane extract gave few compounds like Ethyl hydrogen succinate, Di-n-octyl phthalate and Non-anoic acid etc. When the solvents like Acetone and Acetonitrile were used for standardization, both the solvents were miscible with the toddy samples taken for analysis (Table 1). Since the density of the solvents and Toddy samples were found to be close to each other [7-10].

Results

The major volatile components identified in the fresh toddy were Lupeol and Squalane (Table 2). Propanedioic acid was found in coconut toddy whilst Di-n-octyl phthalate was found in smaller amounts. In Phenyl ethyl alcohol (Figure 1) and 2,4,6,8-Tetraazabicyclo (3.3.0) octan-3-one, 7-nitrimino was volatile components that were found only in fresh coconut toddy the results are shown in Table 3. Thirty one volatile components were identified in coconut toddy from, Thanjavur, Tamil Nadu, India.

Discussion

In this study, only 12 volatile components were identified, whilst Apriyanton et al. [6] reported that around 70 volatile components were identified in coconut toddy.

Toddy of coconut contains a small amount of protein, fat, minerals and vitamins as well as sugar components. These could possibly interact during processing and form some of the volatile components and non-enzymatic browning intermediates as well as Maillard products. (2,5,6,8,9), found glutamic acid, threonine, aspartic acid and serine as major amino acids in fresh sap, whilst proline, methionine, triptophane, and histidine were 4.00 mg/100 g fresh sap [10] reported that toddy of coconut contain sucrose, ash, protein, vitamin C and acids, such as succinic acid, and citric acid (Figure 2).

Conclusion

The volatile components identified in coconut toddy were Lupeol

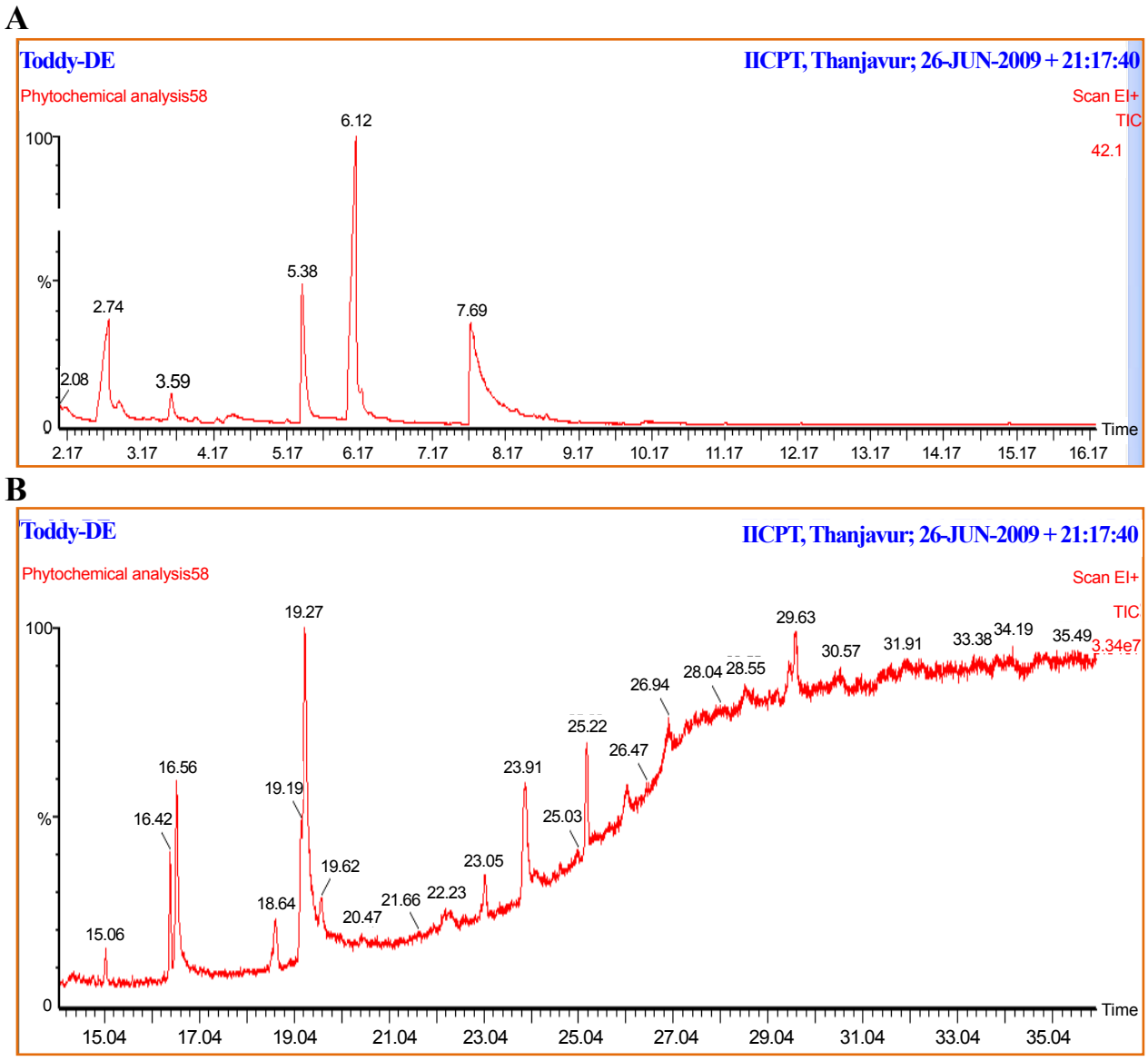

Figure 1: Using Diethyl ether extract.

(Figure 3) and Squalane. The results are shown in Table 2. There were 31 volatile components identified in coconut toddy from this constituency.

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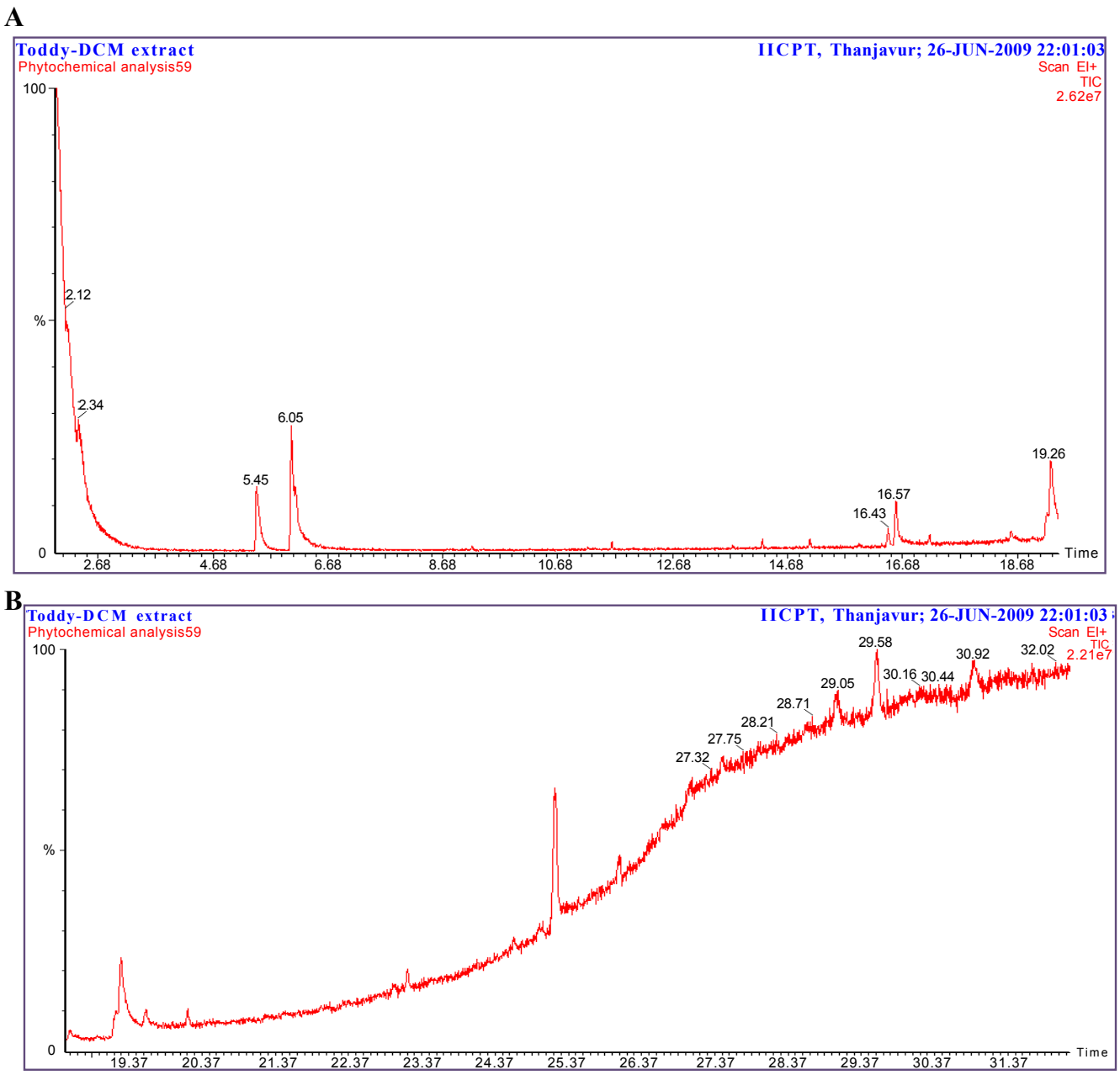

Figure 2: Using Dichloromethane extract.

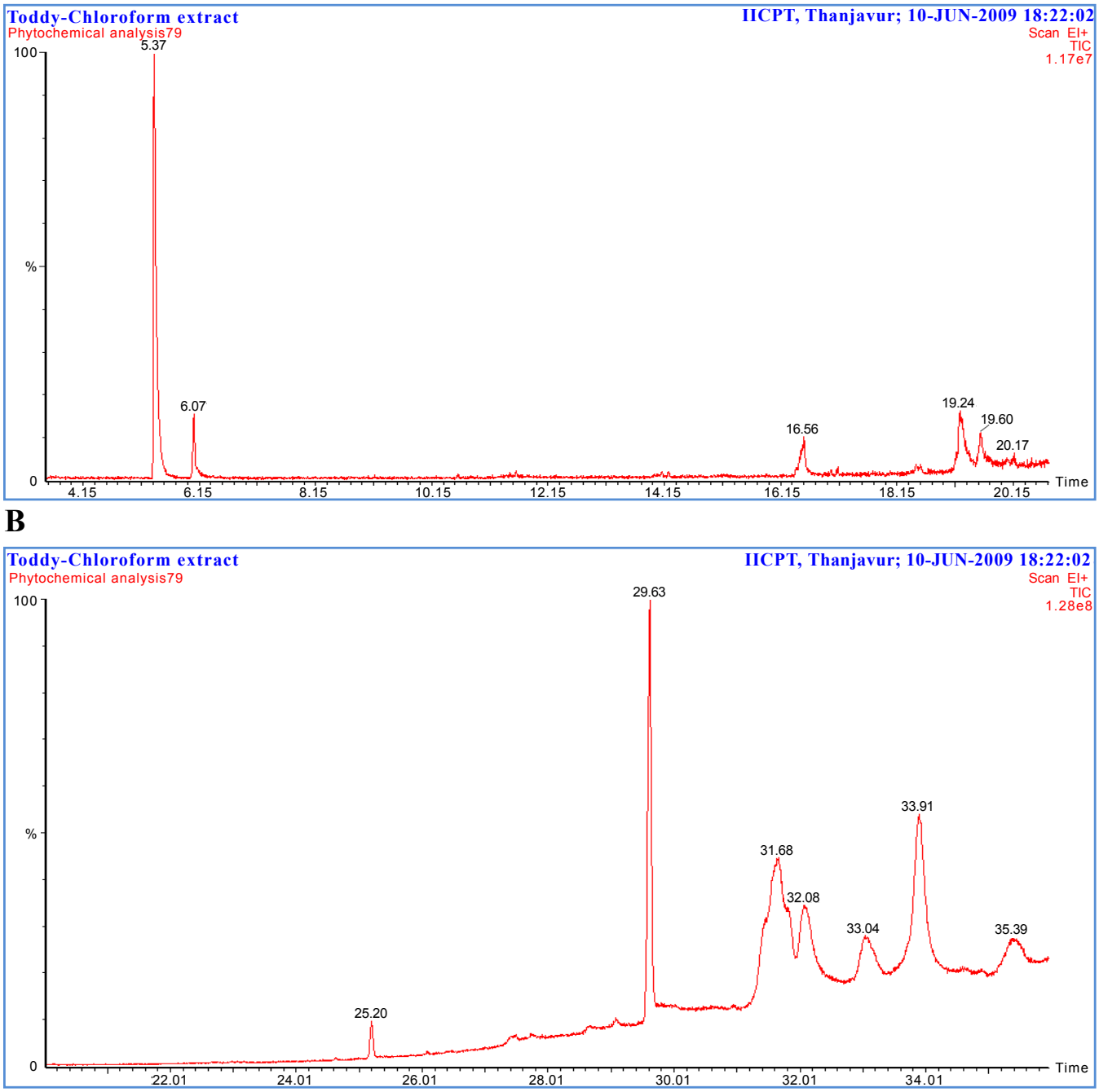

Figure 3: Using Chloroform extract.

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